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PATTERN REGULATION IN AUTOGRAFTS OF *HYDRA OLIGACTIS*: POSITIONAL VALUES AND DIFFUSABLE MORPHOGENS

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ABSTRACT

Grafted *Hydra oligactis* are employed in this study in an attempt to determine if head regeneration in autografts of the species conforms to a positional-information-type control. Two groups of hydras are used: one group has the gastric regions reversed (g-reversal), and a second group with gastric regions and budding regions reversed (gbr-reversal). Regeneration of secondary (2°) heads in the original subhypostomal region of the reversed body regions is observed for both groups at 48, 72, and 96 hours post-grafting. Secondary heads occur in both groups with a significantly greater frequency of 2° heads forming in the gbr-reversal group. These results concur with those seen in "multiply-grafted" hydras wherein tandemly arranged gastric regions are inserted into the grafted animal. Namely, 2° head formation is more frequent at borders located progressively farther from the terminal head. The findings of this study argue strongly for the occurrence of an inhibitory signal (positional information signal) being superimposed upon the graded positional values (for head formation) along the hydra's body column. Furthermore, this study demonstrates that the occurrence of similar results in "multiply-grafted" hydras is a function of intrinsic controls of pattern formation and not an artifactual consequence of abnormal elongation of the animals.

INTRODUCTION

Development of complex organisms with well defined patterns of morphological structure from the modest beginnings of the zygote has long intrigued developmental biologists. Many of the recent models put forth to explain this phenomenon incorporate the concept of cells being endowed with the ability to recognize their neighbors, and to know their position within a tissue field (Wolpert, 1971; Bryant, 1974; French *et al.*, 1976). This type of communication, and subsequent cell-cell interactions render cells non-equivalent and thus programs different developmental commitments by the cells. This concept is termed positional value.

Two of the more popular models purporing to explain the control of pattern formation, namely those of Vernon French and Louis Wolpert, both employ the concept of positional value. French's "polar coordinates" model proposes (French *et al.*, 1976) that cells have positional values which are defined by the cell's position along the circumference of a circle and its position on the radius of that circle. French's model states that regeneration within the circumference of the circle is by intercalary growth between apposing abnormal neighbors. The shortest intercalary route would always be taken during regeneration, even if it means producing a mirror image rather than replacing the missing parts. When all cells have normal neighbors regeneration would cease.

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Wolpert's model (Wolpert, 1971) talks about positional values along some axis, with an inhibitory signal (positional information signal) superimposed upon the positional values. The positional information signal would suppress specific morphogenetic events (such as head regeneration in *Hydra*) by cells that would otherwise perform these acts.

The occurrence of the diffusable inhibitor of head formation, which exists as a gradient decreasing in the direction of the foot, was demonstrated in multiply-grafted *Hydra viridis* (Shostak, 1972, 1973; Shostak and Adams, 1975). Significantly greater frequencies for secondary (2°) head regeneration were observed at graft borders located progressively farther from the terminal head in multiply-grafted animals with three tandemly arranged gastric regions (3g hydras). Since each of the three gastric regions possess theoretically equal morphogenetic competency the gradient in 2° head regeneration was interpreted as resulting from a parallel gradient of the "head inhibitor." Wolpert's model incorporates such an inhibitory substance, existing as a gradient due to "source-sink" activity (Crick, 1970) as depicted in Figure 1. The positional values for *Hydra* would result in a graded disposition toward hypostome and tentacle formation (also depicted in Fig. 1). Cells closer to the head would be more disposed toward head formation.

For the purpose of this study the body regions of *Hydra* have been subdivided into arbitrarily assigned positions representing the relative distance from the head (H). Autografts of *H. oligactis* are employed in this study in an attempt to determine if regeneration of 2° heads conforms to a positional-information-type control.

Materials and Methods

H. oligactis were mass cultured in 20 cm diameter Pyrex dishes in an incubator at 19 ± 0.2°C. The hydras were fed *Artemia salina* nauplii daily and the medium

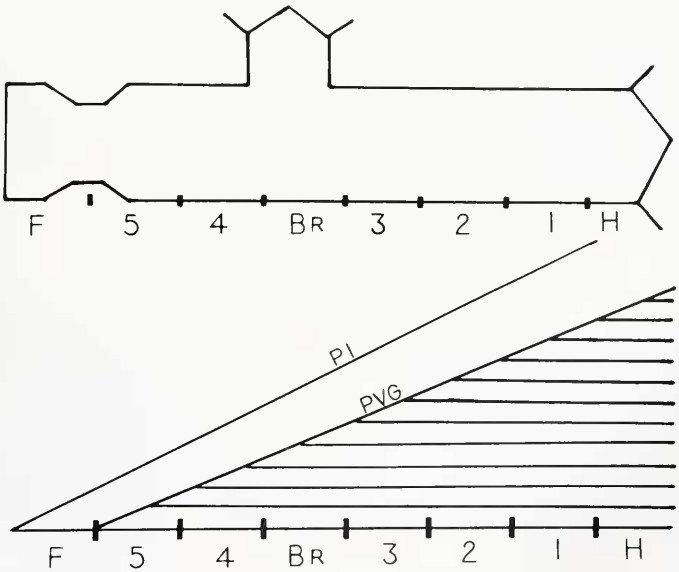

FIGURE 1. Diagram showing the positional value gradient (PVG) and arbitrarily assigned positions H–F for *H. oligactis*. Superimposed upon the positional value gradient is a positional information (inhibitor) gradient (PI).

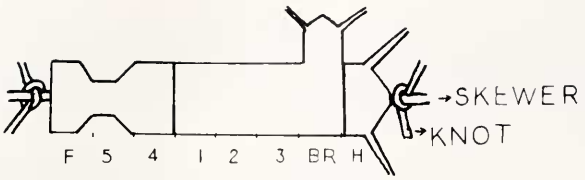

FIGURE 2. Diagram showing an animal that has been transected at the positions I/H border and the positions Br/4 border followed by reversal of the gastric and budding regions (gbr-reversal) and threading of the graft pieces on a skewer of human hair. Two knots of human hair have been placed at either end of the graft pieces to insure apposition during healing.

(artificial pond water, Loomis and Lenhoff, 1956) was changed approximately one hour after feeding to remove uningested nauplii. Experimental animals were kept in 15 mm × 60 mm Petri dishes, one animal per dish, to allow for individual observations of each animal and to prevent any chance of confusing 2° heads with retained buds. All animals used in this study had a single stage I bud (bud staging methods of Shostak *et al.*, 1968 were used).

Grafting was performed by first making the appropriate transections and placing the graft pieces on straight skewers of human hair (Shostak, 1972). Pre-tied knots of human hair were loosened with watchmaker's forceps and placed on each end of the skewer. The knots were then tightened and slid along the skewer until all cut surfaces were touching (Fig. 2). Grafts were allowed to heal for two and one-half hours and were then removed from the skewer. Secondary head regeneration was monitored at 24 hour intervals for a period of four days. Any differences between the frequencies of 2° head regeneration in the groups of animals were tested using Chi square at a 95% confidence level.

RESULTS AND DISCUSSION

Regeneration of 2° heads at the distal end of position 1 is observed at 24, 48, 72, and 96 hours post-grafting. The resulting observations are shown in Table I. No 2° head regeneration is seen at 24 hours for either group of animals, thus 24 hour data are not shown. At 48 hours 18% of the animals in the g-reversal group have

TABLE I

Head regeneration in Hydra oligactis *following reversal grafting*

Treatment	48 Hours	72 Hours	96 Hours
	F, %	F, %	F, %
Gastric region reversal n = 39	7, 18	8, 21	8, 21
Budding region and gastric region reversal n = 39	26, 67 $.001 > P$	28, 72 $.001 > P$	28, 72 $.001 > P$

F: frequency; P: probability of chance deviation between control and experimental frequence (X^2).

regenerated 2° heads. By 72 hours 21% of these animals have regenerated 2° heads and the percentage remains the same at 96 hours. The gbr-reversal group shows 2° head regeneration percentages of 67, 72, and 72% at 48, 72, and 96 hours respectively. The frequency of 2° head regeneration in the gbr-reversal group is higher than that for the g-reversal group at all three time intervals shown in Table I. Figure 3 shows an example of an animal with a 2° head and also a retained bud. This example is chosen to illustrate the point that both due to the relative position and polarity of each, as well as the individual data taken on each animal, 2° heads and retained buds are easily distinguishable. Thus all instances of 2° heads reported here are unquestionably head regenerates.

What the effects of g-reversal and gbr-reversal would be on the relationship between the diffusable inhibitory gradient and the regional positional values along the body column of hydra is seen in Figures 4a and b. Either of the reversal techniques could give the positional value of region one an advantage over the effects of the inhibitory signal thus presenting the possibility of 2° head regeneration. When both the gastric and budding regions are reversed the advantage given the positional value of region one is even greater thus predicting the greater frequency of 2° head regenerates observed for this group.

The results of this study argue strongly for the existence of a head inhibitory signal existing as a gradient along the oral-aboral axis of *Hydra* like that proposed by Shostak (1973) and Wolpert (1969). This study also supports the concept that this head inhibitory gradient is superimposed upon a graded positional value for head formation. Interaction between this position value for head formation and the head inhibitor then are responsible for controlling the pattern of head formation in *Hydra*.

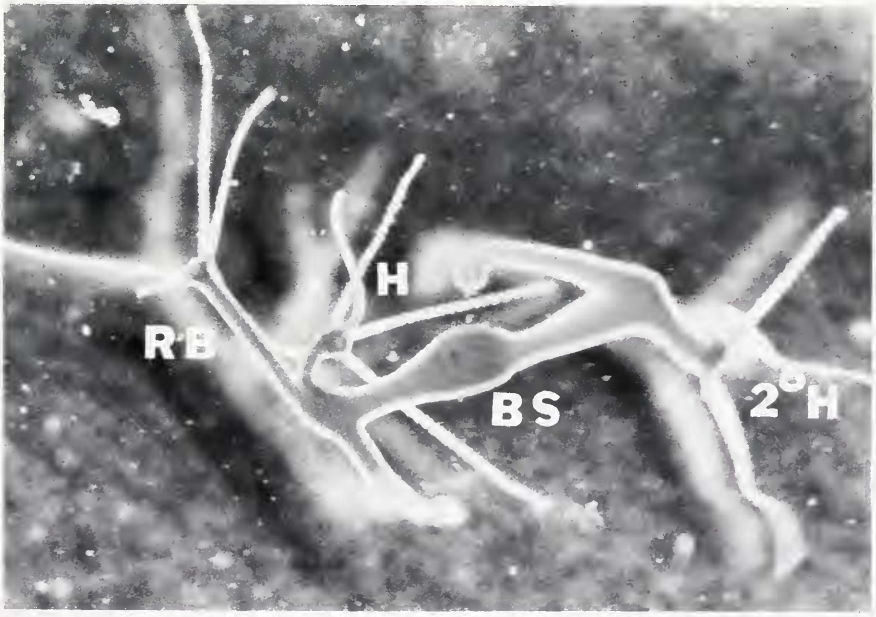

FIGURE 3. This gbr-reversal grafted animal shows a 2° head (2°H), a retained bud (RB), the terminal head of the original animal (H), and the body stalk (BS) of the original animal.

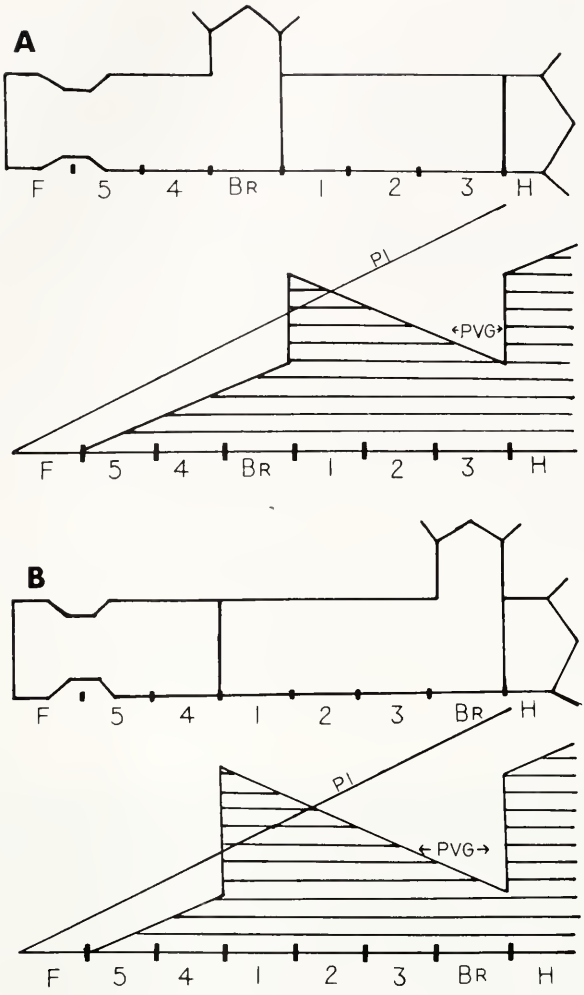

FIGURE 4. Diagrams showing the positional values at points along the hydranth following reversal grafting. Note the greater advantage that position 1 has over the inhibitory signal (positional information) when gbr-reversal is performed (4b) as compared to g-reversal (4a).

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